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☐	L12	L10 and l9	12
☐	L11	L10 and l9 and l8 and l7 and l6	0
☐	L10	beasley-ellen-$.in.	595
☐	L9	francesco-Valentina-$.in.	14
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☐	L7	yan-chunhua.in.	269
☐	L6	Gan-weiniu.in.	45
☐	L5	L3 same antibod$	2
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☐	L3	(glucuronyltransferase adj P) or (GlcAT adj P)	5
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     ANABSTR, ANTE, AQUALINE, BIOENG, BIOSIS, ...' ENTERED AT 16:40:22 ON 16
     MAY 2006
L1          260 S (GLUCURONYLTRANSFERASE (A) P) OR (GLCAT (A) P)
L2            9 S L1 (S) ANTIBOD?
L3            5 DUP REM L2 (4 DUPLICATES REMOVED)

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Marcos I. et al
"Cloning, characterization, and chromosome mapping of the human GlcAT-S gene.";
J. Hum. Genet. 47:677-680(2002).

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J Hum Genet (2002) 47:677–680

SHORT COMMUNICATION

Irene Marcos · José Jorge Galán · Salud Borrego
Guillermo Antiñolo

Cloning, characterization, and chromosome mapping of the human *GlcAT-S* gene

Received: May 23, 2002 / Accepted: September 6, 2002

Abstract We report on the structure, map location, and tissue expression of the human *GlcAT-S* gene. The gene covers approximately 85 Kb on chromosome 6 (6q13) between the *D6S455* and *D6S1673* markers. *GlcAT-S* is composed of four exons and encodes a 324-amino-acid protein, which shows 89% homology with the rat glcat-s protein and is involved in the biosynthesis of the HNK-1 carbohydrate epitope on glycoproteins. Although *GlcAT-S* was considered an interesting candidate gene for the *RP25* locus, the absence of any pathogenic mutations in probands of *RP25*-linked families ruled out that candidacy.

Key words *GlcAT-S* · HNK-1 · Chromosome 6 · *RP25* locus

Introduction

The HNK-1 epitope is present in the extracellular matrix and cell membranes on many different glycoproteins, glycolipids, and proteoglycans. Some of these molecules are implicated in cell–cell and cell–substratum interactions, such as the neural cell adhesion molecule (NCAM), myelin-associated glycoprotein (MAG), L1 protein, transiently expressed axonal glycoprotein-1 (TAG-1), and P0 glycoprotein (McGarry et al. 1983; Kruse et al. 1984; Bollensen and Schachner 1987; Dodd et al. 1988). The HNK-1 epitope is spatially and temporally regulated during the development of the nervous system (Schwarting et al. 1987; Yoshihara et al. 1991). These lines of evidence indicate that the HNK-1 carbohydrate epitope plays crucial roles in cell–cell and cell–substrate interactions such as cell adhesion, migration, and neurite extension. The role of the HNK-1 epitope in the eye may be to structurally stabilize the ciliar body and retina, and to participate in zonular attachments (Uusitalo and Kivela 2001). The HNK-1 epitope is composed of the sulfated trisaccharides sulfate-3GlcAβ1-3Galβ1-4GlcNAc. The key enzymes in the biosynthesis of the HNK-1 epitope are a glucuronyltransferase and a sulfotransferase. The glucuronyltransferase transfers a glucuronic acid (GlcA) in β1-3 linkage to a terminal galactose of Gal β1-4 GlcNAc residue found in different glycoproteins and glycolipids (Chou et al. 1991; Oka et al. 1992). The sulfotransferase enzyme transfers a sulfate to the C-3 position of the GlcA residue (Chou and Jungalwala 1993).

Various glucuronyltransferases have been identified, one for glycoproteins and one for glycolipids, but only one sulfotransferase has been identified for both types of molecule (Ong et al. 1998). Previously, the rat glucuronyltransferases, GlcAT-P (Terayama et al. 1997; Mitsumoto et al. 2000) and GlcAT-D (Shimoda et al. 1999), associated with biosynthesis of the HNK-1 epitope have been identified. GlcAT-P is the major glucuronyltransferase involved in the biosynthesis of the HNK-1 carbohydrate epitope in the rat brain. However, recently, GlcAT-S has been identified as a second enzyme involved in this reaction, although it is expressed in restricted brain regions (Seiki et al. 1999). GlcAT-D and GlcAT-S are the same enzyme on the basis of their cDNA sequences; GlcAT-S was so named because it was the second HNK-1 epitope-associated GlcAT, and GlcAT-D was so named because of its substrate specificity (dual specificity) (Oka and Kawasaki 2002). More recently, the *B3GAT1* gene encoding human GlcAT-P, which is expressed mainly in the brain, has been cloned (Mitsumoto et al. 2000).

Here we report the map location, expression, and genomic organization of the human *GlcAT-S* gene (HGMW-approved symbol, *B3GAT2*).

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Materials and methods

Databases. Database searching and sequence comparisons were carried out using the programs at the U.S. National

Table 1. Polymerase chain reaction amplification of individual exons of the *GlcAT-S* gene

Exon	Forward primers 5′ → 3′	Reverse primers 5′ → 3′	bp	°C
1a	[1]CGCACCCATCACCACTCC	[2]CGGGTCAGCTCCGCTTTC	458	66
1b	[1]GCCCACCATCTATGCCATC	[2]GCAAGGGGCGTGTGACTG	486	66
2	[1]CATTTCCTCCCTCTTTTTC	[2]AGAACAGTCCAGCAGGAAC	296	58
3	[1]AAAAAGAGAAGTACACCAGG	[2]TGAAGGGGAAGGAAATAG	334	57
4	[1]TGTCACCATGAAGAGTGC	[2]CCTAAACTCCAAACATCCTC	252	58

All primers had the universal M13 primers attached to the 5′ end ([1]M13F: cgccagggttttcccagtcacgac and [2]M13R: tttcacacaggaaacagctatgac)

Table 2. The intron–exon structure of the *GlcAT-S* gene

Exon	Exon size (bp)	3′ Intron	Exon	5′ Intron	Intron size (bp)
1	591		ATG AAG . . . CAG GAG	gtaaaggcca	61.566
2	145	ctttttatag	ATG CGA . . . GCA G	gtgagcagtg	31.879
3	149	ctgcactaag	GA TTT . . . ACT AAG	gtattcatta	80
4	87	tccccttcag	GTT CTC . . . GTA TAA		

Upper- and lowercase letters represent nucleotides in the exons and introns, respectively

Center for Biotechnology Information site (NCBI; http://www.ncbi.nlm.nih.gov) and the Japanese "GenomeNet WWW Server" at (http://www.genome.ad.jp). The analysis of the protein sequence was performed using the tools available at the ExPASY Molecular Biology Server (http://www.expasy.ch) and the Baylor College of Medicine (BCM) Search Launcher (http://searchlauncher.bcm.tmc.edu:9331).

Molecular analysis. In order to confirm the intron–exon structure of *GlcAT-S*, the exons were amplified from genomic DNA using primers designed to their intronic flanking sequences (Table 1). The polymerase chain reaction (PCR) products were purified and sequenced as described elsewhere (Marcos et al. 2000).

Expression analysis. Expression analysis was performed using a multitissue Northern blot (Clontech, PaloAlto, CA, USA) and a radioactively labeled cDNA probe (nt 145–420). The cDNA probes were obtained by PCR from Marathon-Ready cDNA of the human retina. PCR conditions included an initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and an extension at 72°C for 1 min. PCR was terminated after final extension at 72°C for 7 min. The primers used were GlcAT-SF (ATGCGAA CCACCCGCAAGGTCTCC) and GlcAT-SR (TCAAT TTTCACTGTGTCCAGGTGG). Hybridization and washing conditions were as recommended by the manufacturer.

Results and discussion

Since the complete coding region of the rat *GlcAT-S* was available from the databases (AB010441), we compared this sequence to those stored in the NCBI and the Japanese GenomeNet databases, using the tBLASTn tool. These searches identified a human expressed sequence tag (EST) clone (AA421030) and two genomic clones (AL121961 and AL450320).

Alignment of the genomic clones and the rat *GlcAT-S* cDNA sequence revealed a genomic organization of four exons spanning approximately 85 Kb of genomic DNA (Fig. 1A). The sequence of the cDNA indicates an open reading frame of 972 bp, encoding a protein of 324 amino acids. *GlcAT-S* introns range between 80 bp and 32 Kb in size, and the gene also has flanking sequences that perfectly match the 5′ and 3′ consensus splice-site sequences (Table 2). A silent transition with a frequency of 0.36 (147C>T) was identified in the molecular analysis of *GlcAT-S* and was confirmed by *BglI* restriction analysis among 50 control subjects. The GlcAT-S transcript appears in the trachea and retina (Fig. 1B). These results are in accordance with the expression of GlcAT-P and GlcAT-S in the rat adult brain or the embryonic brain including the retina (Shimoda et al. 1999; Nagase et al. 2000).

The hydropathy analysis using the TMPred program (http://www.expasy.ch) of the GlcAT-S protein revealed a transmembrane region between amino acids 7 and 25 at the N-terminal end. Next to the transmembrane region, between amino acids 30 and 79, the GlcAT-S protein contains a proline-rich domain. GlcAT-S shows 89% homology with the rat protein, with the highest identity being found in the C-terminal catalytic domain, which contains four highly conserved regions, named motifs I–IV, as previously reported elsewhere (see Fig. 2) (Seiki et al. 1999). The high sequence conservation between the rat and the human *GlcAT-S* strongly suggests conserved functions of this gene in both species. Therefore, the GlcAT-S protein is probably involved in the biosynthesis of the HNK-1 carbohydrate epitope on glycoproteins in the brain.

GlcAT-S is a gene expressed in the retina and located on the long arm of chromosome 6 between the *D6S455* and *D6S1673* markers. *RP25*, a locus for autosomal recessive retinitis pigmentosa, is located in this region (Ruiz et al.

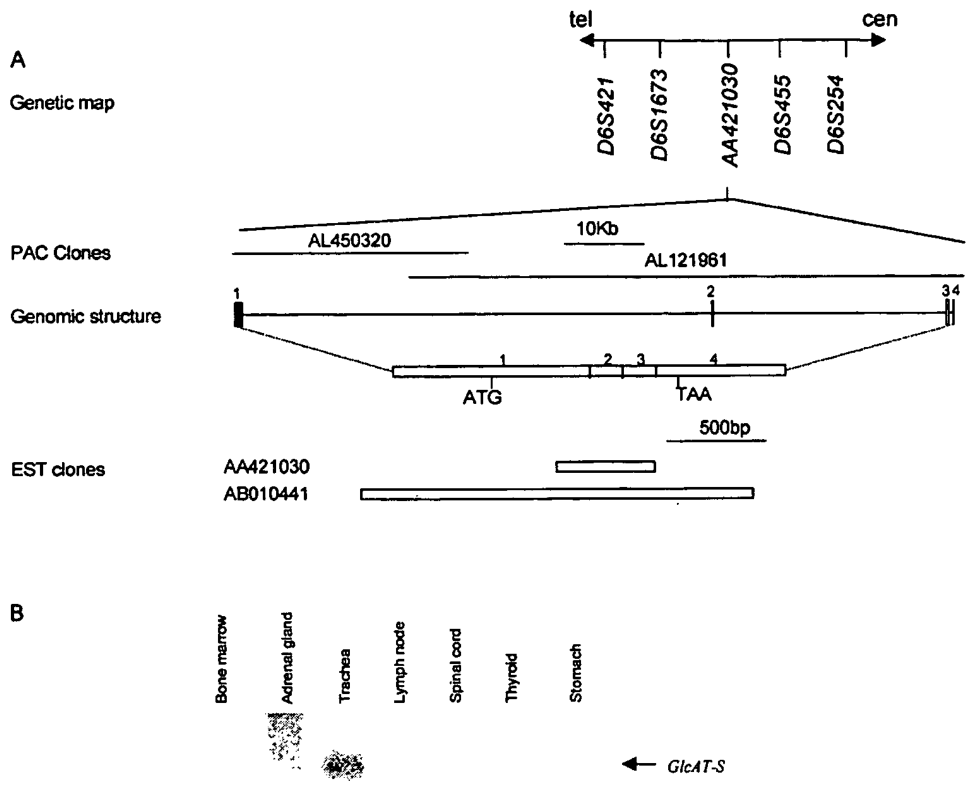

Fig. 1. **A** Physical map and genomic organization of *GlcAT-S*. *GlcAT-S* structure is depicted as a *line* and its exons indicated by *boxes 1* to *4*. The *open bar* under the gene indicates its cDNA with exons. Expressed sequence tag (*EST*) clones are shown below the map. **B** Expression of *GlcAT-S* by a Northern multitissue blot analysis. *PAC*, p1-derived artificial chromosome; *tel*, telomere; *cen*, centromere

Fig. 2. Nucleotide and amino acid sequences of the human *GlcAT-S* gene. Predicted amino acid sequence, a stop codon, a transmembrane domain, *N*-glycosylation sites, and motifs I–IV are shown with *single code letters*, *asterisk*, *box*, *circle*, and *underlining*, respectively

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1   ATGAAGTCCGCGCTTTTCACCCGCTTCTTTATCCTCCTGCCCTGGATCCTAATTGTCATCATCATGCTCGACGTGGAC  78
1   M  K  S  A  L  F  T  R  F  F  I  L  L  P  W  I  L  I  V  I  I  M  L  D  V  D   26

79  ACGCGCAGGCCAGTGCCCCCGCTCACCCCGCGCCCCTACTTCTCTCCCTACGCGGTGGGCCGCGGGGGCGCCCGACTC 156
27  T  R  R  P  V  P  P  L  T  P  R  P  Y  F  S  P  Y  A  V  G  R  G  G  A  R  L   52

157 CCGCTCCGCAGGGGCGGCCCGGCTCACGGGACCCAAAAGCGCAACCAGTCTCGGCCGCAGCCACAGCCGGAGCCGCAG 234
53  P  L  R  R  G  G  P  A  H  G  T  Q  K  R  N  Q  S  R  P  Q  P  Q  P  E  P  Q   78

235 CTGCCCACCATCTATGCCATCACGCCCACCTACAGCCGCCCGGTGCAGAAAGCGGAGCTGACCCGCCTGGCCAACACG 312
79  L  P  T  I  Y  A  I  T  P  T  Y  S  R  P  V  Q  K  A  E  L  T  R  L  A  N  T  104
                                       I
313 TTCCGCCAGGTGGCGCAGCTGCACTGGATCCTGGTGGAGGACGCGGCGGCGCGCAGCGAGCTGGTGAGCCGCTTCCTG 390
105 F  R  Q  V  A  Q  L  H  W  I  L  V  E  D  A  A  A  R  S  E  L  V  S  R  F  L  130

391 GCGCGGGCCGGGCTGCCCAGCACTCACCTGCACGTGCCCACGCCGCGGCGCTACAAGCGGCCCGGGCTGCCGCGCGCC 468
131 A  R  A  G  L  P  S  T  H  L  H  V  P  T  P  R  R  Y  K  R  P  G  L  P  R  A  156

469 ACTGAGCAGCGCAACGCGGGCCTCGCCTGGCTGCGCCAGAGGCACCAGCACCAGCGCGCGCAGCCCGGCGTGCTCTTC 546
157 T  E  Q  R  N  A  G  L  A  W  L  R  Q  R  H  Q  H  Q  R  A  Q  P  G  V  L  F  182

547 TTCGCTGACGACGACAACACCTATAGTCTGGAGCTCTTCCAGGAGATGCGAACCACCCGCAAGGTCTCCGTCTGGCCT 624
183 F  A  D  D  D  N  T  Y  S  L  E  L  F  Q  E  M  R  T  T  R  K  V  S  V  W  P  208
                                       II
625 GTGGGCCTGGTTGGTGGGCGGCGCTACGAACGTCCGCTGGTGGAAAACGGCAAAGTTGTTGGCTGGTACACCGGCTGG 702
209 V  G  L  V  G  G  R  R  Y  E  R  P  L  V  E  N  G  K  V  V  G  W  Y  T  G  W  234

703 AGAGCAGACAGGCCTTTTGCCATCGACATGGCAGGATTTGCTGTAAGTCTTCAAGTCATTTTGTCCAATCCAAAAGCT 780
235 R  A  D  R  P  F  A  I  D  M  A  G  F  A  V  S  L  Q  V  I  L  S  N  P  K  A  260
                                       III
781 GTATTTAAGCGTCGTGGATCCCAGCCAGGGATGCAAGAATCTGACTTTCTCAAACAGATAACAACAGTCGAAGAACTG 858
261 V  F  K  R  R  G  S  Q  P  G  M  Q  E  S  D  F  L  K  Q  I  T  T  V  E  E  L  286

859 GAACCGAAAGCAAATAACTGCACTAAGGTTCTCGTGTGGCACACTCGGACAGAGAAGGTTAATCTAGCCAACGAGCCA 936
287 E  P  K  A  N  N  C  T  K  V  L  V  W  H  T  R  T  E  K  V  N  L  A  N  E  P  312
                                 IV
937 AAGTACCACCTGGACACAGTGAAAATTGAGGTATAA                                           972
313 K  Y  H  L  D  T  V  K  I  E  V  *                                             324
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1998; Marcos et al. 2000, 2001). *GlcAT-S* encodes a protein involved in the biosynthesis of the HNK-1 carbohydrate epitope on glycoproteins. The role of the HNK-1 epitope in the eye may be to structurally stabilize the ciliar body and retina, and to participate in zonular attachments (Uusitalo and Kivala 2001). In addition, an increase in HNK-1 glycoprotein and heparan sulfate proteoglycan synthesis in the inner retinal cells in response to loss of photoreceptors has been observed in various animal models of retinal degeneration (Landers et al. 1994). For these reasons, we considered *GlcAT-S* an interesting candidate gene for the *RP25* locus and decided to perform a molecular study of 18 probands from eight *RP25*-linked families. The four exons of the gene and the corresponding intron–exon boundaries of each patient were amplified using genomic DNA extracted from peripheral blood and intronic primers (Table 1). The PCR products were analysed by enzymatic mutation detection (EMD) (del Tito et al. 1998) and sequenced as previously described (Marcos et al. 2000).

The mutation analysis of *GlcAT-S* among the 18 patients with RP25 did not reveal any pathogenic variants, indicating that this gene is not involved in the pathogenesis of *RP25*.

In summary, we presented the molecular characterization, chromosomal location, and expression of the human *GlcAT-S* gene. This gene, located on the long arm of chromosome 6, within 6q13 and between microsatellite markers *D6S455* and *D6S1673*, is expressed in the retina. The gene encodes a protein involved in the biosynthesis of the HNK-1 carbohydrate epitope on glycoproteins. Although *GlcAT-S* was considered an interesting candidate gene for the *RP25* locus, the absence of pathogenic variations in the patients with retinitis pigmentosa ruled out the gene as responsible for the RP25 phenotype. Nevertheless, the possible implication of this gene in other eye diseases that are mapped to this same chromosomal region remains to be determined.

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